about 92 percent alanine, comparable to its proportion in the reactants. These results indicate a nonrandom incorporation of amino acids (*1*) during thermal polymerization (less strikingly evident from compositions listed in Table 1) and further suggest the presence of a high proportion of glycine in primitive protein (*19*).

Our study has shown that polyamino acids are formed thermally from the proportions of amino acids obtained (*4–8*) by the action of spark discharge, heat, or ultraviolet light on five different geologically plausible atmospheres or hydrospheres. These findings are interpreted to support a proposed molecular evolutionary continuum (*5*), from primitive gases to amino acids to polyamino acids. Our "Miller" and "Sagan" preparations are perhaps the more pertinent in this context, because their syntheses (*4, 8*) apparently yielded free amino acids. However, our "Fox" preparation is also of interest in that the amino acids were obtained [after hydrolysis (*5*)] not from primitive atmospheres of conjectural composition (*1*), but from identified constituents of interstellar space (*20*). Fox and Windsor (*5*) indicate that free amino acids could result naturally from their product [which possibly was hexamethylenetetramine (*20*) and not a polyamino acid (*5*)], and they implicate such amino acids in the evolutionary sequence: ". . . simple compounds → amino acids → proteinoids [polyamino acids] → microspheres. . . ." Our results show that polyamino acids are formed from their reported proportion of amino acids.

The uncertainties concerning the compositions of primitive atmospheres also do not apply to our "meteorite" and "lunar" preparations. The amino acids we used, however, were those quantified after the hydrolysis of samples (*9, 10*). Although some free amino acids have been reported (*10, 21*), the indigenous lunar compounds probably (*22*) existed largely as (unidentified) precursors of amino acids. This study does indicate, however, that if the amino acids ever existed in free form, they could have polymerized thermally. The results, supported by evidence of a thermal lunar history (*23*), suggest that protein-like polymers could exist extraterrestrially.

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Leukemia, Lymphoma, and Osteosarcoma Induced in the Syrian Golden Hamster by Simian Virus 40

Abstract. *Leukemia, lymphoma, and osteogenic and anaplastic sarcomas develop in Syrian golden hamsters inoculated intravenously at 3 weeks of age with simian virus 40, which is a popova virus. Previously, only RNA and herpes DNA viruses have been recognized as capable of inducing leukemia and lymphoma in mammals. The significance of these findings is emphasized in relation to the nature of viral agents that may be involved in analogous diseases of man.*

Avian and murine leukemia and lymphoma are induced by a number of closely related RNA viruses (*1*). However, oncogenic DNA viruses, except those that belong to the herpes group (*2, 3*), have not been shown to possess leukemogenic or lymphomagenic properties. Various workers, therefore, have proposed that RNA viruses will most likely prove to be etiologically associated with the analogous diseases in man. Since this view is based only on negative evidence (*4*), the question of whether a DNA virus of known oncogenic potential other than a member of the herpes group, could induce, under appropriate conditions, leukemia or lymphoma in the experimental animal was investigated. The weanling rather than the newborn Syrian golden hamster and the known oncogenic agent simian virus 40 (SV40), a DNA virus that belongs to the papova (*5*) group, were used.

In this experiment, 300 weanling male Syrian hamsters, 21 to 22 days old, with an average weight of 44 g (range 35 to 55 g; 83 percent weighed 40 to 50 g) were used. Of these, 250 were inoculated via the femoral vein and 50 were held as uninoculated controls. The animals that were inoculated intravenously were divided into four groups (Table 1). Each of 100 received $10^{8.5}$ median tissue culture infective doses ($TCID_{50}$) of SV40 stock virus, strain VA 45-54 (*6*), suspended in 1 ml of culture medium. Each of 50 animals

Table 1. Tumor incidence in hamsters receiving SV40 or SV40 plus specific antiserum. The animals were inoculated intravenously. NRS, normal rabbit serum.

Hamsters (No.)	Inoculum	Tumors (%)
100	SV40	84
50	SV40 + antiserum to SV40	0
50	SV40 + NRS	94
50	Culture medium	0
50	None	0

received 2 ml of a mixture consisting of equal parts of SV40 virus suspension ($10^{8.5}$ $TCID_{50}$/ml) and of undiluted rabbit antiserum to SV40 virus (*7*). Each of 50 received 2 ml of a mixture of equal amounts of SV40 of the same infective titer and of normal rabbit serum (*7*). The remaining 50 animals were each inoculated with 1 ml of culture medium. This medium was prepared from the same components used in the production of the SV40 stock virus in grivet monkey kidney (GMK) cell cultures. It had not been in contact with cells.

Animals were examined (under ether anesthesia) at weekly intervals for tumors. The first tumors were detected 4 months after the experiments were begun. In the groups inoculated with SV40 or SV40 plus normal rabbit serum, the incidence of tumors reached a peak during the fifth month. At this time many of the animals bearing tumors were killed and necropsied. By the end of the 6th month, when all surviving animals were likewise necropsied, the overall tumor incidence was 84 percent in the former and 94 percent in the latter group (Table 1). In the remaining 150 hamsters no tumors were detected either during life or at the time the animals were necropsied at 6 months. These animals included 50 that were inoculated intravenously with the mixture of SV40 and rabbit antiserum to SV40, 50 that received culture medium, and 50 that were held as uninoculated controls (Table 1).

One of the 125 animals with neoplasm had lymphocytic leukemia (Fig. 1), characterized by an elevated peripheral blood leukocyte count (125,000 per cubic millimeter). The leukemic process involved primarily bone marrow, superficial lymph nodes (cervical, axillary, inguinal), spleen, liver, and, to a lesser extent, deep lymph nodes (mediastinal, mesenteric), lungs, intestine, and kidneys. The thymus was intact. Five animals had malignant lymphoma—that is, lymphosarcoma—involving predominantly superficial lymph nodes (Fig. 2) and, to a lesser degree, deep lymph nodes, spleen, and liver. The thymus was spared in all. In two of the five animals, lymphosarcoma cells were identified by indirect immunofluorescence staining for T ("tumor") antigen (*8*) and by the morphology of the cells in the peripheral blood, although no elevated leukocyte counts were recorded.

Of the tumor-bearing animals, 90 (72 percent) had neoplasm that involved mediastinal and mesenteric lymph nodes with frequent tumorous enlargement of the latter. In the majority of cases the liver, spleen, intestine, and Peyer's patches were also infiltrated by malignant cells. The histological features of these neoplasms were consistent either with malignant lymphoma—that is, reticulum cell sarcoma—or, with anaplastic sarcoma. In a large number of these animals, different sites of neoplastic growth or different areas of the same site exhibited slightly different morphology.

Of the tumor-bearing animals, 69 (55 percent) had osteosarcoma. In the majority of cases, the neoplasm arose from bones of the lower extremities. In a few, it arose from bones of the upper extremities or from the ribs. In 36 (52 percent) animals the tumor metastasized to the lungs. Although most of these neoplasms were apparently pure osteogenic sarcomas, some were mixed and consisted of osteosarcoma, spindle-cell sarcoma, and pleomorphic sarcoma. Five other animals had subcutaneous, poorly differentiated sarcoma. It is obvious from the foregoing data that as many as one-third of the animals with neoplasm bore more than one distinct histological type of malignant growth.

The failure of all animals receiving the mixture of virus and specific antiserum to develop tumors argues strongly for the etiological role of SV40 in the induction of the neoplasms found among the animals that had received the virus with or without normal rabbit serum. Additional immunological evidence supporting this conclusion is also provided by the identification of SV40 T antigen (*8*) in parenchymal cells of all 170 primary tumors examined (Figs. 1 and 2B) and by the demonstration of antibody against SV40 T antigen in the serums of a large number of adult hamsters that had received transplants of nine different tumors, representing five distinct morphological types—that is, one lymphocytic leukemia, two lympho-

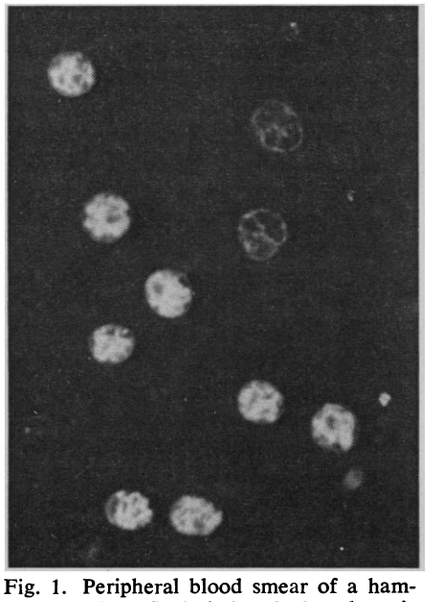

Fig. 1. Peripheral blood smear of a hamster bearing SV40-induced lymphocytic leukemia. The white (reacting) nuclei of the leukemic lymphocytes have been stained by indirect immunofluorescence for the SV40-mediated T ("tumor") antigen. Two normal polymorphonuclear leukocytes are shown with nonreacting nuclei. The erythrocytes are faintly visible; scale 10 μm.

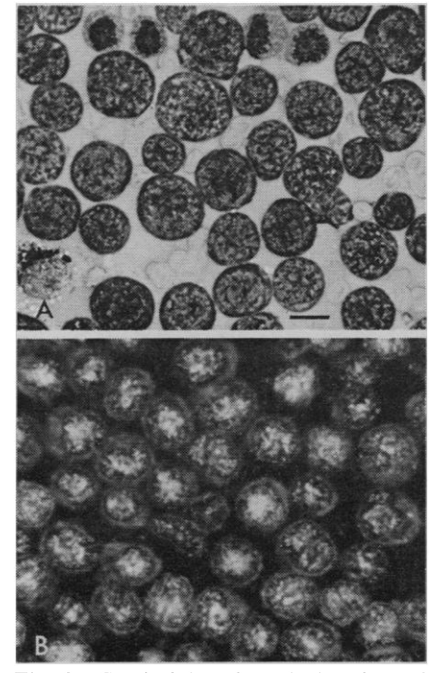

Fig. 2. Cervical lymph node imprints of an SV40-induced hamster lymphosarcoma. (A) Wright's-Giemsa stain; (B) fluorescent staining for SV40 T antigen; scale 10 μm.

sarcomas, two reticulum cell sarcomas, two anaplastic sarcomas, and two osteogenic sarcomas. Finally, since infectious SV40 was not recovered from the cells of two lymphosarcomas, three anaplastic sarcomas, and three osteosarcomas when grown in culture for 1 week in the absence of GMK indicator cells, it appears that the SV40 T antigen is not an expression of a recurrent infectious process, but rather, it is a manifestation of the oncogenic state.

The above data indicate that, under appropriate experimental conditions (*9*) relating both to the host (species, age) and to the viral agent (dosage, route of inoculation), the DNA virus SV40 can induce leukemia, lymphoma, and osteosarcoma in addition to the anaplastic sarcoma with which it is already known to be associated. These findings do not support the view held by some investigators that viruses which induce experimental leukemia, lymphoma, and osteosarcoma are always of the RNA type (*1, 10*). It should be of great interest, therefore, to determine whether other oncogenic DNA viruses (polyoma virus, adenoviruses) can induce, under comparable experimental conditions, hematopoietic, lymphoreticular, and osteomesenchymal malignant neoplasms. This may well prove to be the case, since evidence has been presented which suggests that polyoma virus may rarely cause osteosarcoma in the mouse (*11*), and that herpes DNA viruses can induce avian (*2*) and simian (*3*) lymphomatous proliferations. Furthermore, there is some evidence suggesting that a herpes-type virus, the Epstein-Barr agent (*12*), may be related etiologically to Burkitt's lymphoma, a neoplasm that affects predominantly children living in Africa (*13*).

Although a decision on the issues raised should await the results of further inquiry, we can now state with assurance that, for the first time, a DNA virus other than a member of the herpes group has been implicated in the experimental induction of leukemia, lymphoma, and osteosarcoma. It is evident, therefore, that, in attempting to isolate and identify viral agents possibly involved in analogous diseases of man, attention should be directed toward DNA viruses.

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Hormone-Calcium Interactions with the Plasma Membrane of Rat Liver Cells

Abstract. *The binding constants and the number of binding sites for insulin, glucagon, epinephrine, cyclic adenosine monophosphate, and calcium ions for the plasma membrane of rat liver were determined by Scatchard plots. The plots are biphasic or multiphasic, an indication of at least two types of binding sites for each ligand. At least three types of binding sites were found for insulin. In the concentration range of 10^{-6} to 10^{-8} molar, glucagon, epinephrine, and hydrocortisone increased calcium ion binding to the plasma membrane, whereas insulin decreased this binding. At hormone concentrations of 10^{-6} to 10^{-7} molar, glucagon was the most effective in increasing calcium binding, but at a hormone concentration of 10^{-8} molar, hydrocortisone was the most effective in stimulating calcium binding. Adenosine triphosphate reversed the effect of insulin and inhibited the effect of the other hormones. These studies suggest a relation between hormones and calcium with respect to membrane structure and function.*

Certain hormones, such as glucagon, epinephrine, and insulin, probably exert at least part of their physiological efects at the level of the cell plasma membrane (*1–4*). Rasmussen (*5*) has proposed a model to integrate the relation between hormone, calcium ion, and cyclic adenosine monophosphate (cyclic AMP). In this model, certain hormones act to stimulate the membrane-bound adenylate cyclase that produces cyclic AMP from adenosine triphosphate (ATP). The cyclic AMP then activates protein kinases in the cytoplasm which, in turn, phosphorylate several enzymes or contractile proteins within the cell to modulate the activities of these macromolecules. A second controlling influence in the cell is postulated to be the calcium ion (Ca^{2+}). The mobilization of calcium from intracellular pools, or its influx from extracellular fluid, are thought to occur by hormonal or electrical stimulation of the cell plasma membrane or by an action of cyclic AMP on intracellular membranes. The increased intracellular concentration of Ca^{2+} may induce other enzyme reactions within the cell, or it may act as a negative feedback control on adenylate cyclase. Calcium pumps are also considered to play a role in the active efflux of calcium from the cell. Rasmussen (*5*) suggests